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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Sharat Singh	Examiner: Bradley L. Sisson
Serial No.: 09/466,369	Art Unit: 1655
Filed: December 15, 1999	
For: Individually Addressable Surfaces For Multiplexed Operations	**RESPONSE TO OFFICE ACTION**

Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

This response is submitted in response to the Office Action mailed May 1, 2000, the time period for response having been extended by the attached Petition for Extension of Time and the Requisite Fee. Reconsideration and re-examination are requested.

The Examiner is respectfully requested to make the following amendments.

AMENDMENTS

To the claims:

Claim 1, line 4, claim 3, line 2, and claims 5 and 6, line 1, each occurrence change "moieties" to --sites--.

REMARKS

CERTIFICATE OF FIRST-CLASS MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first-class mail in an envelope addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231 on 31 August 2000.
(Date)

(Signature)

SARA GARCIA
(Printed Name)

In view of the above amendments and the following remarks, the Examiner is respectfully requested to allow claims 1-18, the only claims pending in this application, and pass this application to issue. The rejections will be taken up in seriatim.

Paragraph 2. Claim Rejections – 35 U.S.C. Sec. 112

The rejection of claims 1-6 is avoided by the proposed amendments to claims 1, 3, 5, and 6. These amendments are corrections of drafting errors that caused confusion due to incorrect antecedence. The amendments correct antecedence to phrases in the preamble.

Paragraph 4. Claim Rejections – 35 U.S.C. Sec. 103

In paragraph 4, claims 1-18 were rejected under 35 USC 103(a) as unpatentable over Still (Acc. Chem. Res. 1996, 29:155) in view of Benson *et al.* (U.S. Pat. No. 6,051,719), Rothman *et al.* (U.S. Pat. No. 4,921,878), and Heller (U.S. Pat. No. 4,824,776).

The present invention is concerned with creating individual addresses for solid surfaces for the purpose of improving the level of multiplexing possible in a single mixture. Improving multiplexing relies upon establishing as broad a variety of labels as possible that are readily distinguished. In the claimed invention multiplexing is increased by using lanthanide dyes that yield accurate, quantitative readouts without quenching or energy transfer. When dyes with these characteristics are employed, multiplexing can be dramatically increased because both *combinations* of different dyes and their relative *proportions* can serve as variables in the address. Using both combinations and proportions as a means of increasing multiplexing, as well as the utilities and advantages of this approach to multiplex labeling, has been previously unappreciated.

Dyes that are subject to significant quenching and energy transfer are not suitable to the present invention because these reactions will reduce quantum yield and broaden emission spectra. As a result, the quantitative dynamic range of detection is reduced, and accurate measurement of differing *ratios* of dyes is not feasible. Furthermore, spectrum broadening will reduce resolution within a mixture of dyes located on a single particle. Both quenching and energy transfer prevent an accurate quantitative measurement of the composition of a dye

combination, and limit multiplexing capacity to the number of individual, spectrally resolvable dyes that are available. These characteristics are factors that have limited the level of multiplexing obtained with methods making use of organic dyes.

The Examiner cites Still as disclosing the use of solid supports, or beads or particles, which are labeled with chemical moieties. These moieties are used in combinations to create labels that encode a particle associated with an individual member of a chemical library. The method as disclosed by Still is limited to qualitatively determining the presence or absence of a particular chemical moiety, and does not teach the advantages to multiplexing by monitoring the *ratios* of chemical moieties in a label. As such, the level of multiplexing enabled by the methods of Still is limited to 2^N different labels from N different moieties (page 158, column 2, lines 1-3). The present invention discloses means for detecting both the combination *and ratios* of dyes composing an individual address. This dramatically increases the degree of multiplexing possible from a fixed number of different dyes. For example, with two label molecules called A and B, the methods of Still will provide for a multiplex of four (no label, A, B, 1A+1B). The level of multiplexing possible with the methods of the present invention is theoretically infinite, as the following combinations can be distinguished: 1A, 1B, 1A+1B, 1A+2Bs, 1A+3Bs, *etc*, 2As+1B, 3As+1B, *etc.*

Not a limitation

Benson *et al.* is cited by the Examiner as disclosing the use of multiple color fluorophores in the context of an automated DNA sequencing method, wherein each of four dyes used represents a specific nucleotide base. This reaction is conducted as a multiplex to generate sequence information for all four nucleotides in a single sample. DNA sequence information is obtained by generating a ladder of bands in a sequencing gel, each band yielding a fluorescent signal from the single dye that terminates chain elongation at that position in the sequence. While the sequencing reaction is effectively a multiplex of four individual reactions, the fluorescent signals generated from each band in the gel arise from a *single* fluorescent species, and as such, the method as disclosed provides for a very restricted level of multiplexing. The advantages of using dyes in combination on a single entity are not taught. The sequencing of *one* template from individual dye signals can be achieved because the required set of four organic dyes with resolvable emission spectra is available. However, a multiplex of eight, required for

sequencing two nucleic acid templates, is not currently possible using organic dyes. The methods disclosed in the present invention would allow monitoring many separate sequencing reactions in a single mixture.

The Examiner cites Rothman *et al.* as disclosing a list of fluorophores used in a variety of assay formats (column 10, lines 25-32). The Examiner cites this list in light of our disclosure, however it includes only one class of labels that is appropriate to the present invention (that of the lanthanide chelates), along with several others that are not useful in the present invention, including fluorescein, rhodamine, and umbelliferone (all organic dyes, which are subject to both the quenching and energy transfer reactions discussed above). Rothman *et al.* do not teach the advantages or particular properties of the dyes they list, and therefore their listing cannot be construed as a suggestion for the use of lanthanide dyes in particular. Furthermore, Rothman's disclosure does not suggest improved means of multiplexing, nor does it teach the use of dyes in combinations. In the present invention, lanthanide dyes are employed for overcoming severe multiplexing limitations encountered with the other types of dyes listed by Rothman *et al.*

There are additional characteristics of lanthanide dyes (as compared to organic dyes) that confer previously unappreciated advantages for quantitative, multiplexed labeling. These dyes absorb within a narrow spectrum, therefore a larger multiplex can be monitored using a single excitation laser. Lanthanide dyes emit over a broader range of frequencies and have very sharp emission lines, which facilitate resolution of multiple dyes within a mixture. Furthermore, the present invention discloses methods for labeling particles by dyeing them, which confers much greater flexibility in controlling dye combinations and ratios than can be obtained by chemical reaction.

The Examiner cites Heller as disclosing the use of a variety of fluorescent compounds, including lanthanide complexes, as useful in his invention (column 5, lines 25-27). Heller's list includes radioactive, colorimetric, and fluorescent labels, including fluorescein, Texas Red, Lucifer Yellow, pyrene, lanthanide complexes, etc. (column 5, lines 16-27). However, as pointed out with respect to the Rothman *et al.* citation, all but the lanthanide dyes disclosed by Heller are not useful in the present invention, and this citation should not serve as prior art simply in light of our disclosure.

Heller's invention is concerned with improving the sensitivity of hybridization assays, and he suggests the possible use of more than one fluorophor per probe *in order to improve detection* (column 5, lines 29-33). Specific methods for improving detection are not taught, so it is not possible to know the meaning of this statement. Several interpretations are possible, including increasing signal strength by attaching multiple molecules of a single type of dye to each probe, creating a unique signal through a Stokes shift by attachment of a pair of dyes that allow energy transfer, or differentiating species in a multiplex by using dye combinations. Heller does not specify conditions or dye combinations. His disclosure does not suggest a multiplexed system of quantitatively differentiated dye concentrations, nor does it reasonably suggest the use of multiple dyes in combinations in a single particle. The use of and advantages resulting from quantitatively measuring a combination of fluorophores present in different ratios within individual particles are not taught.

Dependent claims 2-6 incorporate all the subject matter of claim 1 and add additional subject matter, which makes them *a fortiori* and independently patentable over Still, Benson *et al.*, Rothman *et al.*, and Heller. Similarly, dependent claim 8 in relation to claim 7, and 11-18 in relation to claim 10 are *a fortiori* and independently patentable over Still, Benson *et al.*, Rothman *et al.*, and Heller.

CONCLUSION

In view of the above amendments and remarks, it is submitted that this application is in good and proper form, and now ready for allowance. Examiner is respectfully requested to withdraw the rejections and pass this application to issue. If in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney collect at (650) 328-4400.

Respectfully submitted,

Dated: August 30, 2000

Barbara Rae-Venter, Ph.D.
Reg. No. 32,750

Rae-Venter Law Group, P.C.
P.O. Box 60039
Palo Alto, CA 94306
Telephone: (650) 328-4400
Facsimile: (650) 328-4477

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